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DESERT BIOME

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1971 PROGRESS REPORT

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ABSTRACT

Laboratory feeding experiments were conducted in an endeavor to determine the digestive rate in desert pupfish using ^{32}P in rams horn snails as the tagged food material. Feeding and sacrifice intervals were done over a period of 96 hours at a temperature of approximately 22°C. Initial results indicated a turnover of activity values around 16 hours, the time at which digestion was essentially completed. A repetition of the experiment with emphasis on sampling along the time interval up to 16 and including 24 hours resulted in a curve, indicating digestion as occurring up through 24 hours with no turnover of values from the gastrointestinal tract to flesh. Although ^{32}P transfer to the flesh was observed, no effort was made to determine the kinetics of exchange to the cardiac muscle, liver or blood. Further refined investigations made over a wider range of temperatures and the consideration of certain factors (isolation, time of feeding, temperature stress) which promote or hinder digestive processes are necessary to indicate the apparent relationship between uptake and transfer as related to general digestive rate.

INTRODUCTION

A common method of estimating the degree of digestion which has occurred in organisms is to dissect and visually observe stomach contents. This estimating process is done over given time periods. Much of the accuracy needed in estimating digestive rate in this manner may vary due to the inadequacy, unrepresentativeness or unavailability of past data dealing with a specific animal type or group and its food habits. If existing data are available, the visual estimates of the degree of digestion may have varied simply because of the inherent differences which result when different observers are involved. Estimates of digestion rate may also vary when different food items or a conglomerate is encountered. The errors of estimations may be even greater when population groups of an identical species which have differing dietary preferences are encountered.

In an attempt to circumvent the possible differences in observer error, a preliminary experiment was conducted utilizing ^{32}P as a radioactive tracer. This initial attempt was primarily an effort to determine if ^{32}P would serve as a digestion indicator and thereby warrant further experimentation. Therefore, the primary purpose of the research reported here was to determine if transfer of ^{32}P in labelled snails would occur from the gastro-intestinal tract into the flesh and therefore serve as an index of digestive rate in the desert pupfish, *C. nevadensis*.

METHODS & PREPARATION PROCEDURES

All desert pupfish used in this experiment were caught in minnow traps from Saratoga Springs, Death Valley National Monument, California. These fish were held in two 10-gallon aquariums equipped with gravel bottom filtration systems and an ultraviolet light source. Fish were kept on a diet of commercially prepared food (tradename, TETRA-MIN) and the room temperature where the colony was kept was maintained at an almost constant 22°C with an average photoperiod of nine hours. For the experiment, an algal culture was started and maintained in a separate aquarium for later feeding to snails. Snails used in this experiment were of the common rams horn variety and these served as the intermediate trophic level between algae and fish.

Prior to feeding of labelled snails, it was necessary to determine equilibrium of snails with algae and water. Experimental procedures involved the dilution of an initial concentration of ten millicuries of $Na_2H^{32}PO_4$ contained in a one milliliter volume to ten milliliters using triple-distilled water. The activity at any given time (A_t) of the original radioisotope was calculated by formula

$$A_t = A_o\, e^{-nt}$$

Experimental procedure involved the adminstering of a known amount of radiotracer into a known volume of water containing the three components. Samplings of water, algae and snails were done over specific time periods to establish uptake and retention curves. Algae and snail equilibrium curves indicated rapid uptake by both components, the former attaining equilibrium in the first hour and the snails in 15 to 24 hours. A second experiment was followed using phosphorous-deprived components, algae and snails which were held and acclimated in distilled water for a period of approximately two weeks. These components were then placed in distilled water of a known volume and isotope administration was followed by time interval sacrifices.

Since radiation substances which are chemically identical with normal tissue constituents will generally be metabolized in exactly the same way as their non-radioactive counterparts (^{32}P as compared to elemental phosphorous), both will have the same uptake and distribution. If equilibrium has taken place, the amount of stable element which is normally present is related to the amount of radioisotope observed in tissue. A tissue which normally contains

much phosphorous would be expected to take up a lot of radioactive tracer under controlled conditions where the only available phosphorous is in easily identifiable tracer form. Results from this latter study indicated that phosphorous-deprived algae and snails tended to accumulate and retain more of the available tracer material when it was the only source present as compared to a situation where both a radioactive and an elemental counterpart were present. Further discussion will be covered in the results section of this report.

All of the fish used in this experiment were individually isolated in 2000 milliliter beakers where they were acclimated and starved for a period of 15 to 20 hours. The preparation of equilibrated snail, used as tracer food, follows the same procedure described in a prior report. Snails were shelled and the amount of fleshy parts to be fed were weighed to the nearest 0.01 gram. After feeding was observed, sacrifice followed after given time periods and samples were prepared by separating the entire gastrointestinal tract, including the gills, from the rest of the body. The two sub-components from each sample were then converted into a homogeneous and identical physical state by the use of concentrated nitric acid. This wet ashing technique was done in four-inch diameter stainless steel planchets. Since the sample size was small to begin with, no dilution to a given volume was necessary for an aliquot. Each sample was placed under infra-red lamps and evaporated to dryness. Samples containing residue after evaporation were further ashed by addition of more nitric acid until a uniform layer was observed on the planchet.

The radioactivity present in each sample was measured for gross beta by gas proportional counting. The instrument used was a Beckman WIDE-BETA system equipped with an automatic sample changer. The efficiency of this instrument (ratio of observed counts to known disintegration) by using a ^{90}Y - ^{90}Sr standard source was 52 percent and the average background count rate was 5.2. The counting time interval for all fish samples was 50 minutes.

RESULTS

96-Hour Experiment

The equilibrium curve for the initial experiment (see Figure 1 and Table 1) indicates rapid uptake by algae during the first hour after isotope administration. The activity level attained after the first hour remained consistent throughout the 168-hour sampling period. Snail uptake was observed as a gradual increase in time with equilibrium being reached at about the 20th hour. Though uptake was noted, this activity was not truly representative of uptake by grazing on algae. Throughout the sampling period, snails were not observed to be grazing. It is assumed that the uptake by snails occurred primarily by exchange of body fluids with that of the surrounding water. Though the degree of uptake did not occur as expected, the time interval at which equilibrium was attained was established for the subsequent feeding period.

Individual fish were placed in 1000 ml beakers containing triple-distilled water and starved for approximately 15 hours, a period which was also sufficient for acclimation. Tracer material was fed to each fish and sacrificing was done over specified time periods. Data presented in Table 2 and Figure 2 are expressed as corrected activity in counts per minute per gram of material. Results indicate a fairly rapid transfer of ingested tracer material occurring from the gastrointestinal tract (to be referred to hereafter as G.I. tract) to flesh. The initial five-minute sample result for both the G.I. tract and flesh showed a total of 4855 counts per minute. A gradual decrease in total activity was noted over time; the 96-hour sample had a sum of 235 counts per minute for both components. This decrease in activity over time is a result of both exchange and excretion processes. Sample activity values for flesh increased over time and the resultant decrease in activity values for flesh was noted as digestive processes transferred the tracer material. As noted in Figure 3, activity for both components gradually merged until values were about identical at about the 16th hour. The proceeding sample results for periods of 48, 72 and 96 hours showed a decrease in activity values with neither component showing any observable accumulation over time. Thus, after digestion is completed with transfer to the flesh, most activity remaining is residual in nature and organs showing affinity for the tracer material retain their full complement,which is gradually eliminated over time.

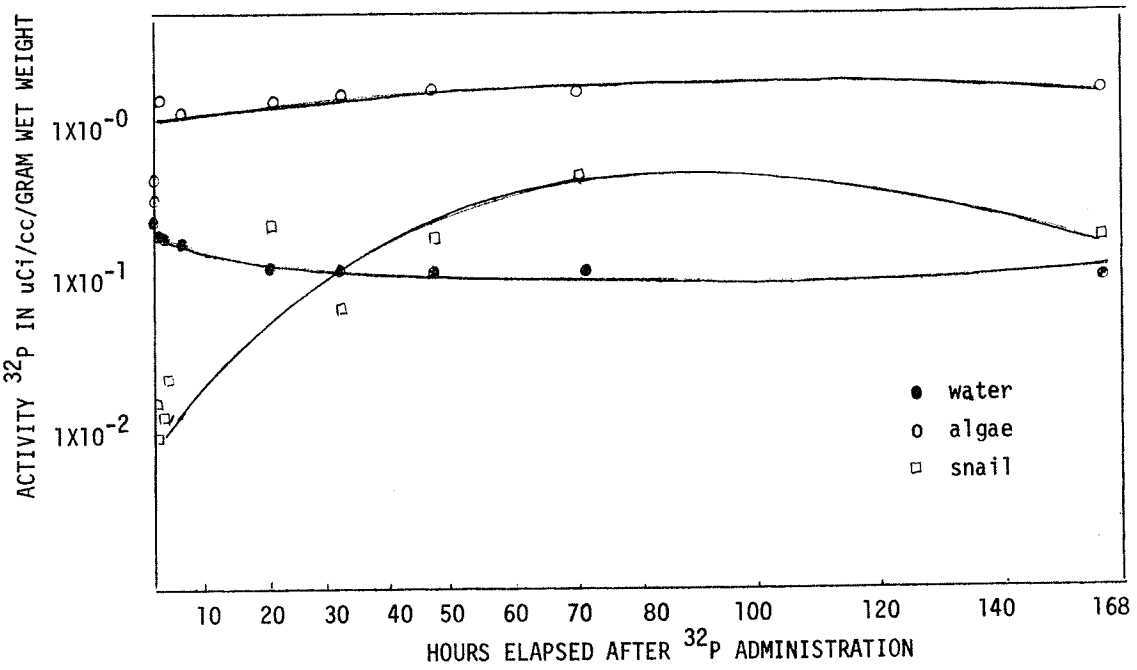

Figure 1. Behavior of ^{32}P in an aquarium microecosystem.

Table 1. Uptake and concentration of ^{32}P by algae and snails in an aquarium microecosystem.

Time Elapsed	Activity μCi/Gram		
	Water	Algae	Snail
5 minutes	2.52×10^{-1}	3.52×10^{-1}	9.89×10^{-3}
20 minutes	2.27×10^{-1}	4.70×10^{-1}	2.56×10^{-2}
1 hour	2.20×10^{-1}	1.89×10^{-0}	1.64×10^{-2}
4 hours	1.97×10^{-1}	1.49×10^{-0}	6.01×10^{-2}
22 hours	1.52×10^{-1}	1.86×10^{-0}	2.83×10^{-1}
30 hours	1.40×10^{-1}	1.98×10^{-0}	9.32×10^{-2}
47 hours	1.29×10^{-1}	2.23×10^{-0}	1.98×10^{-1}
72 hours	1.20×10^{-1}	2.10×10^{-0}	3.23×10^{-1}
168 hours	1.06×10^{-1}	2.26×10^{-0}	1.61×10^{-1}

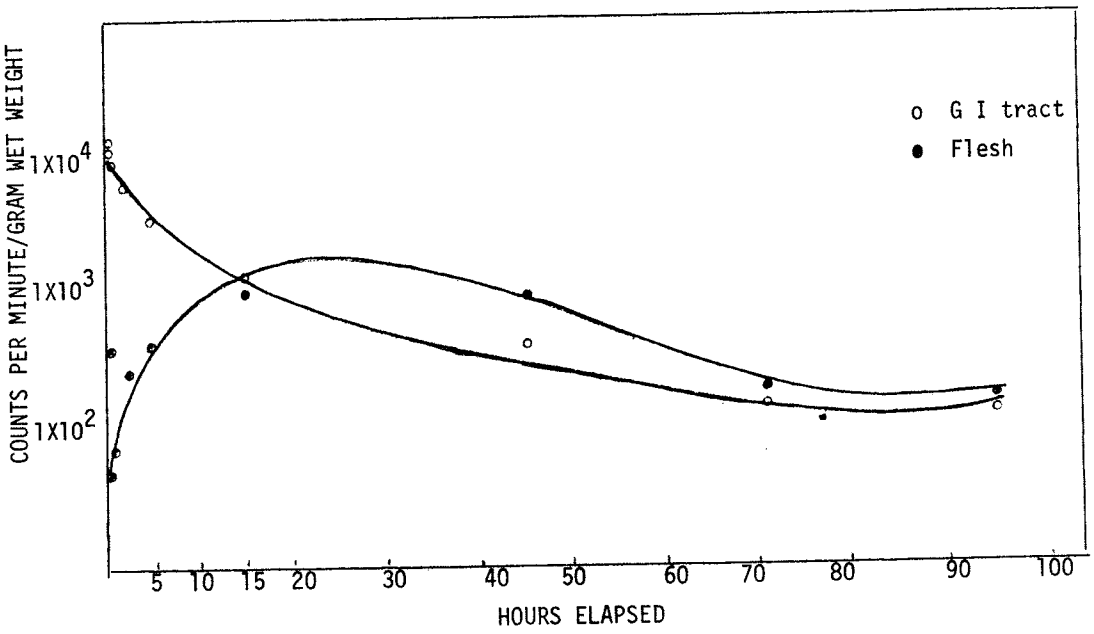

Figure 2. Retention of ^{32}P in the gastrointestinal tract vs. flesh of *C. Nevadensis* over specified time intervals.

Table 2. Retention of ^{32}P in the gastrointestinal tract vs. flesh of *C. nevadensis* over specified time intervals.

Elapsed Time	Weight in grams		Activity Expressed in cpm		Activity Corrected to cpm/gram	
	G.I. Tract	Flesh	G.I. Tract	Flesh	G.I. Tract	Flesh
5 min.	0.385	1.375	4778	77	12,410	56
20 min.	0.350	2.150	3710	174	10,600	81
1 hr.	0.599	1.820	4953	836	8.268	459
4 hr.	0.292	1.187	1929	350	6,606	295
8 hr.	0.235	1.392	866	598	3,685	430
16 hr.	0.400	1.050	408	822	1,020	782
24 hr.	0.386	1.625	Bkgd.	10		6
48 hr.	0.420	2.185	249	668	306	899
72 hr.	0.458	2.220	31	229	103	71
96 hr.	0.250	1.300	37	198	152	198

24-Hour Experiment

A 24-hour experiment was performed to determine primarily the reproducibility of results under the area of the retention curve which included the shorter time intervals. Since initial investigation indicated digestion being completed at 16 hours after feeding, it was felt that experimentation with an increased number of samples taken from zero time up to 16 and including 24 hours would better characterize the digestive curve. An equilibrium study was again performed, this time using phosphorous-deprived algae and snails. Both components were kept in distilled water over a period of approximately two months. Deprivation of phosphorous from algae and snails was done in an effort to determine:

1. Whether the uptake rate for both components could be increased appreciably under controlled conditions.

2. Whether the uptake rate for both components would result in a longer period of time before equilibrium was attained.

Phosphorous deprivation resulted in a greater concentration of activity to snails over a period of 120 hours than in the initial experiment where undeprived snails were sacrificed after 168 hours. The concentration factor for undeprived snails after 168 hours was

$$\text{snails/water} = \frac{1.61\text{x}10^{-1}\ \mu\text{Ci/gr}}{1.06\text{x}10^{-1}\ \mu\text{Ci/ml}} = \underline{1.52}$$

Two concentration factors for phosphorous-deprived snails after 120 hours:

$$\text{snails/water} = \frac{1.61\text{x}10^{-1}\ \mu\text{Ci/gr}}{1.76\text{x}10^{-4}\ \mu\text{Ci/ml}} = \underline{306.3}$$

Phosphorous deprivation also resulted in a retention curve which was not clearly defined in interpreting equilibrium. A rapid uptake is noted for both components up until the 20th hour. The uptake is then gradual with no indication of an equilibrium constant.

In the subsequent 24-hour feeding of snails to fish (Figure 4 and Table 4), the data are presented as activity corrected to cpm/gram of material over time. Inital activity results of the G.I. tract from the successive sacrifice periods of five minutes through ten hours indicate greater than 96.8% to 99.7% retention. The 12, 16 and 24-hour sacrifices show G.I tract retention levels of 92.9%, 85.7% and 89.7% retention, respectively.

The initial five-minute sacrifice showed 460 cpm in the G.I. tract and 25 in flesh, a total expressed as 485 cpm total fish. The following ten-minute sacrifice showed 1332 cpm total fish, or 847 counts greater than the initial sacrifice. A summation and comparison of activity corrected to cpm/gram for the five and ten-minute sacrifices indicate total counts of 2347 for the former and 7145 cpm for the latter, a higher value than would have been expected if all snails were equilibrated. The same anomalies are noted for other sacrifice periods where total cpm/gram values are dispersed over wider than normally expected ranges. Activity ranges observed in the flesh component are extremely low, indicating digestion as not occurring over the observed 24-hour period. The maximum corrected activity noted in flesh occurred for the four-hour sacrifice period where 55 cpm/gram was measured. This value is 2.2% of the total noted in both flesh and G.I. tract components.

Though an uptake curve is difficult to define, loss of total activity was noted over time. After the tenth hour sacrifice and including 12, 16 and 24 hours, a resultant decrease in total activity values occur. Actual counts as observed are drastically lower than earlier time period sacrifices, though percentages still suggest most of the ingested material as remaining in the G.I. tract.

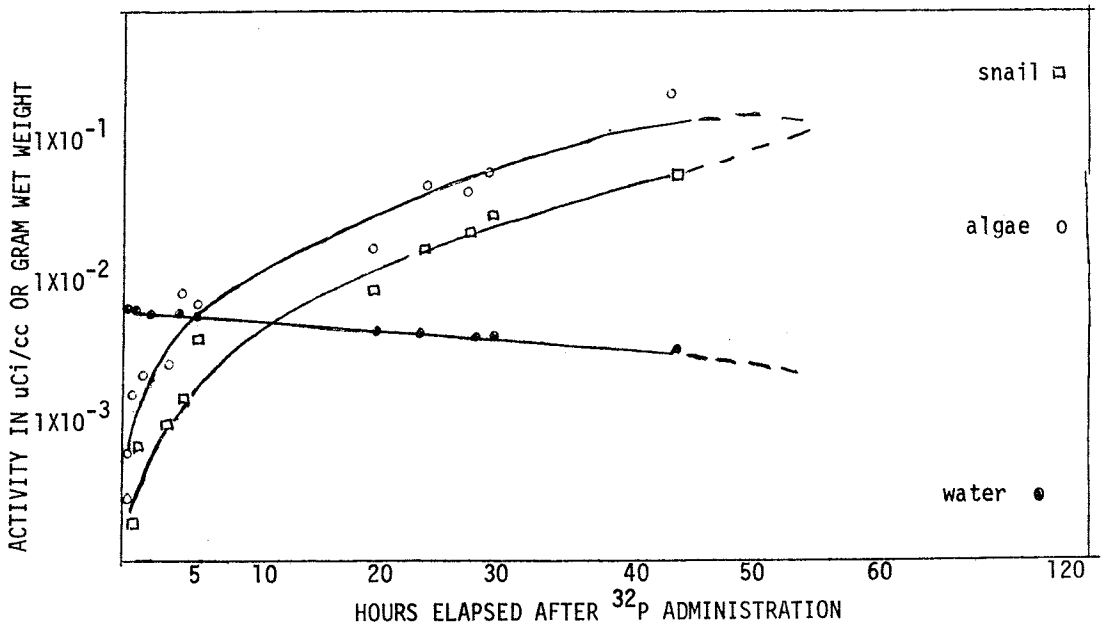

Table 3. Uptake and concentration of ^{32}P by phosphorous-deprived algae and snails in an aquarium microecosystem.

Time Elapsed	• Water µCi/cc	° Algae µCi/cc	Snails µCi/cc
5 minutes	$6.74x10^{-3}$	$3.36x10^{-4}$	$5.53x10^{-5}$
10 minutes	$6.73x10^{-3}$	$4.93x10^{-3}$	$9.07x10^{-5}$
20 minutes	$6.62x10^{-3}$	$1.30x10^{-3}$	$1.53x10^{-4}$
1 hour	$6.65x10^{-3}$	$2.38x10^{-3}$	$5.20x10^{-4}$
2 hours	$6.64x10^{-3}$	$2.62x10^{-3}$	$6.77x10^{-4}$
4 hours	$6.31x10^{-3}$	$9.97x10^{-3}$	$1.23x10^{-3}$
(5-35') 8 hrs.	$6.21x10^{-3}$	$7.79x10^{-3}$	$4.33x10^{-3}$
(21-15') 16 hrs.	$5.01x10^{-3}$	$2.36x10^{-2}$	$9.31x10^{-3}$
24 hours	$4.80x10^{-3}$	$4.83x10^{-2}$	$1.74x10^{-2}$
28 hours	$4.63x10^{-3}$	$4.22x10^{-2}$	$2.01x10^{-2}$
(29-30') hrs.	$4.50x10^{-3}$	$5.02x10^{-2}$	$2.39x10^{-2}$
(45-15') 48 hrs.	$3.72x10^{-3}$	$1.52x10^{-1}$	$4.09x10^{-2}$
120 hours	$1.76x10^{-4}$	$3.59x10^{-2}$	$2.45x10^{-1}$

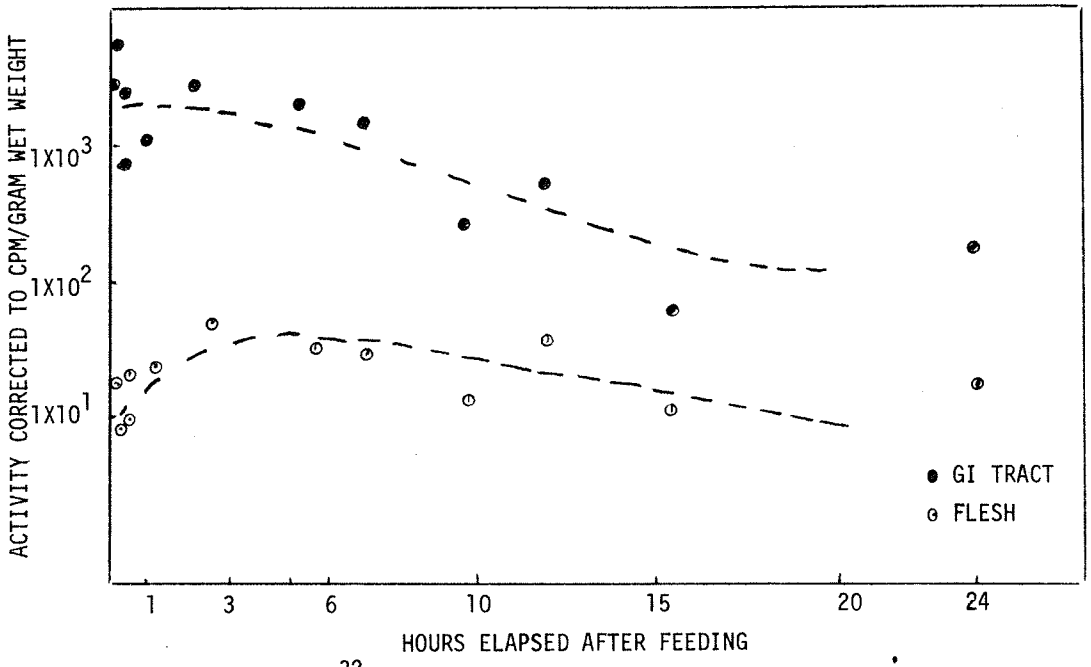

Figure 4. Retention of ^{32}P in the gastrointestinal tract vs. flesh.

Table 4. Retention of ^{32}P in the gastrointestinal tract vs. flesh of *C. nevadensis* over specified time intervals.

Order No.	Elapsed Time	Weight in Grams		Activity Expressed in cpm		Activity Corrected to cpm/gram	
		G.I. Tract	Flesh	G.I. Tract	Flesh	G.I. Tract	Flesh
1	5 min.	0.198	1.04	460.0	25.0	2323.0	24.0
2	10 min.	0.185	1.20	1320.0	12.0	7135.0	10.0
5	45 min.	0.22	0.89	143.0	10.0	650.0	11.0
6	1 hr.	0.19	0.85	422.0	22.0	2221.0	26.0
7	2 hrs.	0.22	1.38	241.0	39.0	1095.0	28.0
10	4 hrs.	0.21	1.01	513.0	55.0	2443.0	55.0
11	6 hrs.	0.21	0.94	434.0	39.0	2066.0	42.0
15	8 hrs.	0.28	0.95	480.0	29.0	1714.0	31.0
17	10 hrs.	0.20	0.81	60.0	8.0	300.0	10.0
19	12 hrs.	0.19	0.70	84.0	24.0	442.0	34.0
21	16 hrs.	0.12	0.94	5.0	7.0	42.0	7.0
26	24 hrs.	0.15	0.98	17.0	13.0	113.0	13.0

DISCUSSION

The effects of phosphorous deprivation in the microecosystem resulted in a higher concentration factor in snails than in previous experiments.

The results of the equilibrium curve also indicate a rapid uptake with equilibrium not being attained during the experimental time span of 120 hours. Though equilibrium of snails was not attained, multiple snail sacrifices at a given time before feeding to fish indicated the mean concentration value of snails at any point in time. In the 96-hour feeding experiment, undeprived snails which were used attained equilibrium at about the 20th hour. The equilibrium curve (Figure 1) maintained a flat plateau throughout the period following the 20th hour. All snails observed had essentially attained a steady state of equilibrium with the surrounding medium. The observed responses of the two separate sub-samples of snails can be attributed primarily to phosphorous deprivation in one sub-sample. Also the feeding behavior in the phosphorous-deprived sub-sample resulted in a higher concentration factor, 306.3 times greater than the surrounding medium as compared to 1.52 times greater in snails which were non-grazing. As previously mentioned, the non-grazers attained equilibrium primarily by bodily fluid exchanges with the surrounding medium. The anomalies observed in the 96-hour feeding experiment (especially total counts observed in both sub-components of G.I. tract and flesh), can be attributed to the feeding activity in individual snails. The presented data show a total of 2347 counts per minute/gram on the five-minute sample fish and 7145 counts per minute/gram in the ten-minute sample. Other sampling periods along the 24-hour scheme also show wide variations in total counts observed. Initial multiple snail samplings to determine mean activity values in time series did not indicate wide variation in results to warrant rejection of data. It is reasonable to postulate that the feeding behavior of *C. nevadensis* greatly affected the total activity observed along the 24-hour sampling scheme.

C. nevadensis has a habit of manipulating food in its mouth. The observed feeding behavior is to take the food in its mouth, chew on it for a while, blow it out into the surrounding medium, swallowing the small acceptable bits and repeating the process until all or most of the food is ingested. As noted, the 24-hour sampling scheme was done with snails which fed on algae prior to being sacrificed and fed to fish. The radioactive tracer which concentrated in algae is further concentrated in snails by the action of their grazing. The algae which is ingested by snails contributes to the higher concentration factor. In this case, bodily exchange processes with the surround medium are neglibible, as compared to snail uptake where grazing is observed. The feeding habit of *C. nevadensis* results in the breakup of the snails' G.I. tract which contains ingested algae, the major contribution of the tracer material. In its feeding behavior, *C. nevadensis* utilizes bits of snail flesh and softer organs as food but loses most of the tracer algal material in a cloud of minute particles when it manipulates and blows the food material into its surrounding medium. Repetition of this feeding behavior results in ingestion of the snail flesh with considerable loss of tracer material. Comparison of early time series feeding results with data observed in 12, 16 and 24-hour results indicates a greatly reduced total count. The reason for this anomaly is not apparent, especially when transfer is not observed into the flesh components.

Differences in rate of sorption between organisms could result from differences in trophic level position. Peak concentrations could have been attained in snails without the use of algae as an intermediary, as noted in the 96-hour sampling and feeding scheme where grazing on algae by snails did not occur. Still, a degree of equilibrium was atained in flesh. This equilibrium between flesh and the surrounding medium proved to result in data which had less variation and greater reliability in data interpretation. The values presented in the 96-hour uptake and transfer studies thus serve as relevant evidence that digestion is essentially completed in 16 hours. The evidence, though scanty, is adequate for drawing a conclusion from the data.

The organism used in the present study could be used in further uptake studies with variations to account for effects of temperature, photoperiod, time of feedings, etc.